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PATENT
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Michelle P. Chicos — Printed name of person mailing correspondence

Michelle P. Chicos — Signature of person mailing correspondence

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Tsuyoshi Tokusumi et al.	Art Unit:	1648
Serial No.:	09/702,498	Examiner:	Shanon A. Foley
Filed:	October 31, 2000		
Title:	PARAMYXOVIRUS VECTORS USED FOR TRANSFER OF FOREIGN GENES		

Assistant Commissioner For Patents
Washington, D.C. 20231

DECLARATION OF YOSHIYUKI NAGAI

I declare:

1. I am a co-inventor, along with Tsuyoshi Tokusumi, Akihiro Iida, Mamoru Hasegawa, of the subject matter described and claimed in the above-referenced patent application.

2. I am a co-author on the Kato *et al.* (*J. Virol.* 73:9237-9246, 1999) publication. Any description of the invention described and claimed in the above-referenced patent application or in any continuation or divisional application thereof in this publication was the contribution of one of the present inventors, Yoshiyuki Nagai, alone, notwithstanding the inclusion of the additional authors, namely Atsushi Kato, Katsuhiro Kiyotani, Mohammad K. Hasan, Tatsuo Shioda, Yuko Sakai, Tetsuya Yoshida, who were merely working under the direction of Yoshiyuki Nagai, providing technical assistance. These additional authors did not contribute to the conception and/or reduction to practice of the invention disclosed and claimed in the above-referenced application or in any continuation or divisional application thereof.

3. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patents issued thereon.

Date: 14, March, 2002

Yoshiyuki Nagai

(2)

#11

PATENT
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Tsuyoshi Tokusumi et al.	Art Unit:	1648
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Title:	PARAMYXOVIRUS VECTORS USED FOR TRANSFER OF FOREIGN GENES		

Assistant Commissioner For Patents
Washington, D.C. 20231

DECLARATION OF YOSHIYUKI NAGAI

I declare:

1. I am a co-inventor, along with Tsuyoshi Tokusumi, Akihiro Iida, Mamoru Hasegawa, of the subject matter described and claimed in the above-referenced patent application.

2. I am a co-author on the Tokusumi *et al.* (*Abstracts of The Third Annual Meeting of the American Society of Gene Therapy*, Program No. 890, 2000) publication. Any description of the invention described and claimed in the above-referenced patent application or in any continuation or divisional application thereof in this publication was the joint contribution of the present inventors alone, notwithstanding the inclusion of the additional author, namely Atsushi Kato, who was merely working under the direction of the present inventors providing technical assistance. This additional author did not contribute to the conception and/or reduction to practice of the invention disclosed and claimed in the above-referenced application or in any continuation or divisional application thereof.

3. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patents issued thereon.

Date: March 28, 2002

Yoshiyuki Nagai

(3)

#11

PATENT
ATTORNEY DOCKET NO. 50026/025001

Certificate of Mailing: Date of Deposit: June 4, 2002

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Michelle P. Chicos — Printed name of person mailing correspondence

Michelle P. Chicos — Signature of person mailing correspondence

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Tsuyoshi Tokusumi et al.	Art Unit:	1648
Serial No.:	09/702,498	Examiner:	Shanon A. Foley
Filed:	October 31, 2000	Customer No.:	21559
Title:	PARAMYXOVIRUS VECTORS USED FOR TRANSFER OF FOREIGN GENES		

Assistant Commissioner for Patents
Washington, D.C. 20231

DECLARATION OF AKIHIRO IIDA UNDER 37 C.F.R. §1.132

I declare:

1. I am an inventor of the subject matter described and claimed in the above-captioned patent application.

2. I have read the Office Action mailed December 4, 2001. I have also read the following references: Conzelmann EP 0 702 085 A1 and Conzlemann US Pat. No. 6,033,886.

3. In addition to the results described in the above-captioned specification, and in further support of the observation that its insertion site in the Sendai virus genome can determine the expression level of a foreign gene, I submit the following experimental data.

4. In this series of experiments, following the teaching of the above-identified specification, expression from a foreign gene in recombinant Sendai virus was determined as follows.

5. A novel NotI site was inserted between the stop codon and the end signal of the L gene of cDNA of the Sendai virus (SeV) full-length genome, pSeV(+)(see Example 1 of the present U.S. Patent Application (Serial No. 09/702.498)). Specifically, as shown below in Figure 1, pSeV(+) was digested with EcoRI, resulting fragments were separated by electrophoresis, and a band (5010 bp) was excised, purified and recovered by QIAEXII Gel Extraction System (QIAGEN). The fragment was subcloned into pBluescript II SK+ (STRATAGENE) by ligation. Next, the QuikChange Site-Directed Mutagenesis kit (STRATAGENE) was used to introduce an additional NotI site. Primers used for the introduction were as follows: a sense primer: 5'-cgtgcagaac gatcgaagct ccgcggccgc tggaagtctt ggacttgtcc-3', an antisense primer: 5'-ggacaagtcc aagacttcca gcggccgcgg agcttcgatc gttctgcacg-3'.

After the introduction of a NotI site, the plasmid was digested with Xho I and Mlu I, a resulting fragment (2010 bp) was purified and recovered similarly, and assembled to cDNA of the full-length Sendai virus genome (Fig. 1).

6. Figure 1 shows construction of a Sendai virus vector having a Not I site inserted into the viral genome.

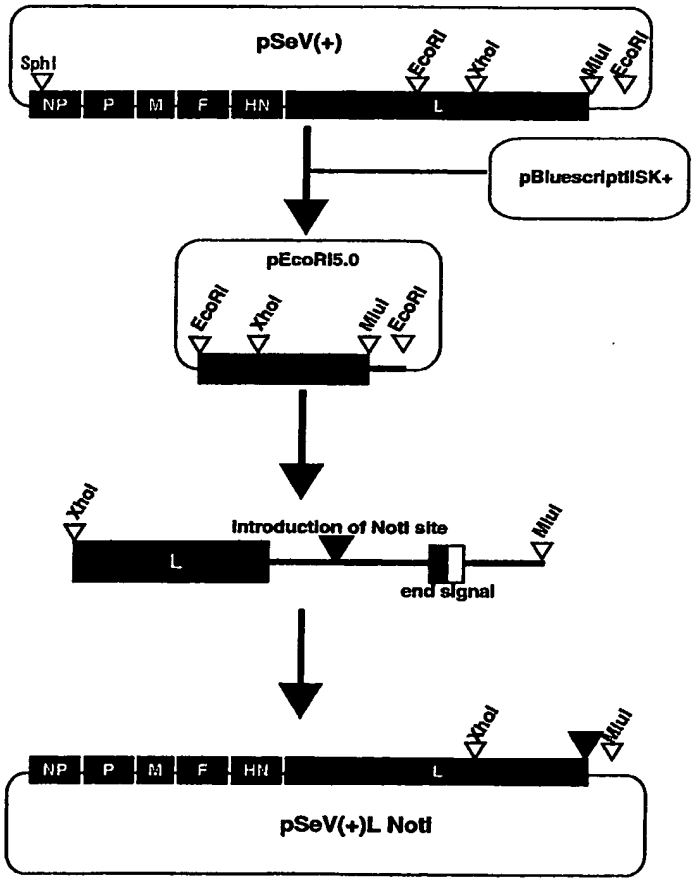

Figure 1

7. Human vascular endothelial growth factor (hVEGF) gene was subcloned by PCR as a reporter gene for evaluating the expression level of a foreign gene. For the primers, a 5' primer containing AscI restriction site: 5'-gcggcgcgcc aaccatgaac tttctgctgt cttgggtgca ttgg-3', and a 3' primer: 5'-gcggcgcgcc tcaccgcctc ggcttgtcac atctgcaagt-3' were synthesized, and PCR amplification was performed. KOD-plus-DNA polymerase (TOYOBO) was used. Following PCR, amplified products were digested with AscI, and purified and recovered using electrophoresis. In order to add a sequence unit of end signal-intervening sequence-start signal of Sendai virus to the hVEGF fragment, the following construction was performed (Fig. 2). MluI-SphI fragment (3317 bp) of pSeV18$^+$ (US Patent Application No. 09/702.498, Example 1) was inserted into LITMUS38 to make pAC114. A synthetic double-stranded DNA comprising end signal-intervening sequence-start signal and a multicloning site (AscI-SwaI) [sense strand: 5'-ggccgctaag aaaaacttag ggtgaaagtt cacttcacga gggcgcgccg tttaaactgc-3', antisense strand: 5'-ggccgcagtt taaacggcgc gccctcgtga agtgaacttt caccctaagt ttttcttagc-3'] was incorporated into the NotI site of pAC114 to make pAG180 (Fig. 2). The purified/recovered PCR product encoding hVEGF was ligated into the AscI site of this plasmid and cloned. After digestion with NotI, the hVEGF gene fragment was recovered and purified by electrophoresis and ligated into the NotI site of the Sendai virus genomic cDNA

constructed above. This viral vector cDNA was named pSeV+L/VEGF. Separately, end signal-intervening sequence-start signal (US Patent Application No. 09/702.498, Example 1 and Fig. 2) was ligated to the downstream of hVEGF cDNA, and was introduced into pSeV18+ or pSeV(+)HNL (US Patent Application No. 09/702.498, Example 1) to make pSeV18+/VEGF or pSeV(+)HNL/VEGF, respectively. These constructs were used for the comparison analysis described below. Recombinant Sendai viruses were reconstituted with pSeV+L/VEGF, pSeV18+/VEGF and pSeV(+)HNL/VEGF by using the method described in Example 1 of US Patent Application No. 09/702.498, and recovered (named SeV18+/VEGF, SeVHNL/VEGF, and SeVL/VEGF, respectively).

8. Figure 2 shows the generation of a hVEGF reporter construct containing end signal - intervening sequence - start signal of Sendai virus.

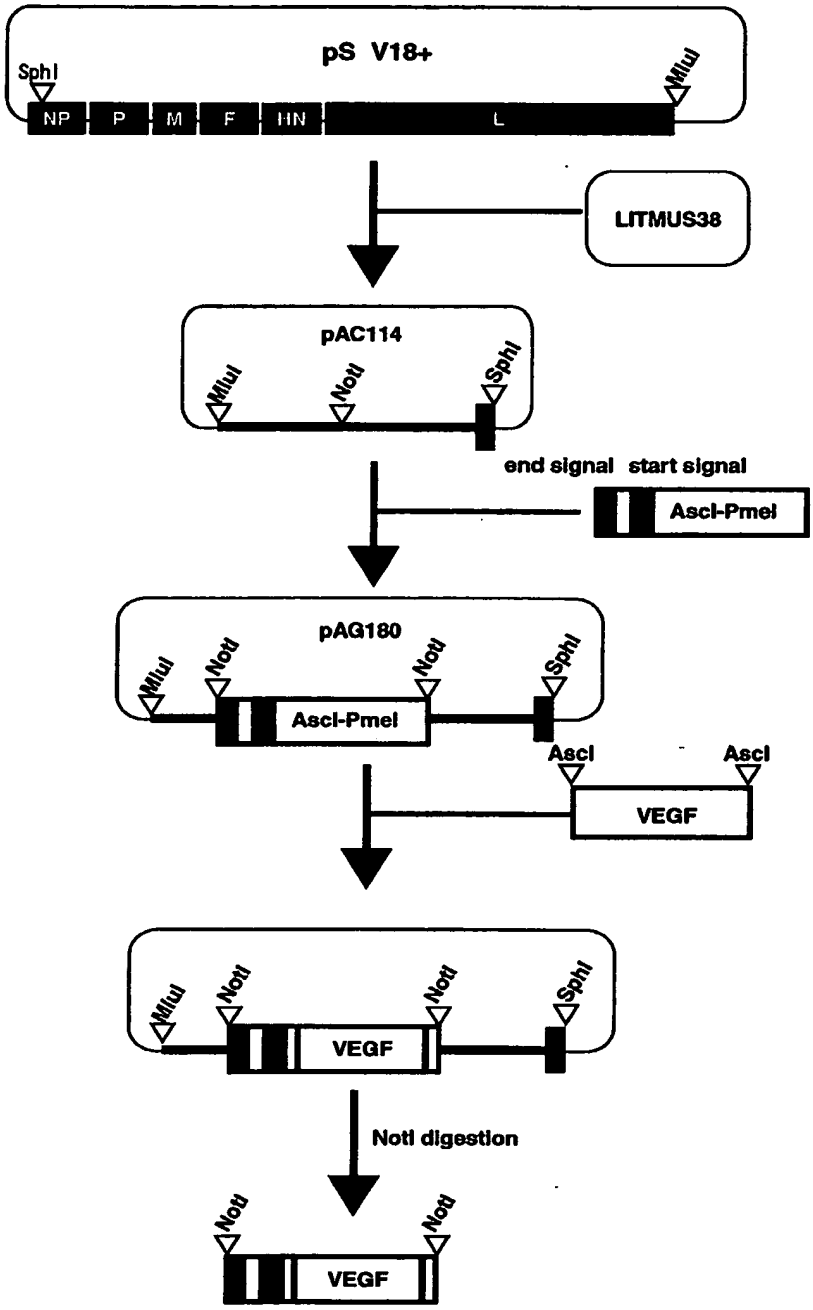
pS V18+
SphI
MluI
NP
P
M
F
HN
L
LITMUS38
pAC114
MluI
NotI
SphI
end signal start signal
AscI-PmeI
pAG180
MluI
NotI
NotI
SphI
AscI-PmeI
AscI
AscI
VEGF
MluI
NotI
NotI
SphI
VEGF
NotI digestion
NotI
NotI
VEGF

Figure 2

9. It was shown, as follows, that reporter gene expression is affected by the location of the reporter gene within the Sendai virus genome. LLC-MK2 cells were seeded onto a 6-well-plate at 5×10^5 cells per well, cultured for 24 hours, infected with each viral vector at a multiplicity of infection of 5. Culture supernatant (200 μ l) was collected after a 24-hr culture, and VEGF assay was conducted by ELISA. The result is shown in Figure 3. It shows that the Sendai virus having a foreign gene between the L gene and the trailer sequence is capable of expressing the foreign gene at 1/10th of the level of when a vector having the foreign gene upstream of the NP gene is used.

10. Figure 3 shows that reporter gene expression varies depending on the location of the reporter within the Sendai virus genome.

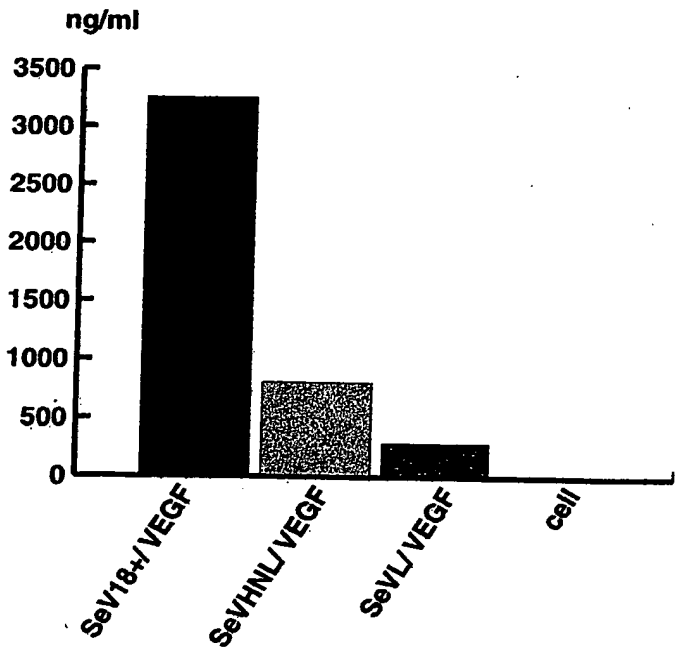

Figure 3

11. In view of these experimental results, which were obtained using the teachings of the 09/702,498 application, I submit that not only expression of the SEAP gene described in the 09/702,498 application, but also of the hVEGF gene, is dependent on the position of the gene within the Sendai virus genome. Accordingly, the 09/702,498 application teaches that the expression level of a foreign gene may be predicted based on its insertion site in the Sendai virus genome. In contrast, the Conzelmann et al. patent applications do not teach how to predict the expression level of a foreign gene inserted into a Sendai virus.

12. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patents issued thereon.

June 3, 2002
Date

Akihiro Iida